Supplementary information

Chemical Modulation of the Biological Activity of Reutericyclin: a Membrane-Active Antibiotic from *Lactobacillus reuteri*

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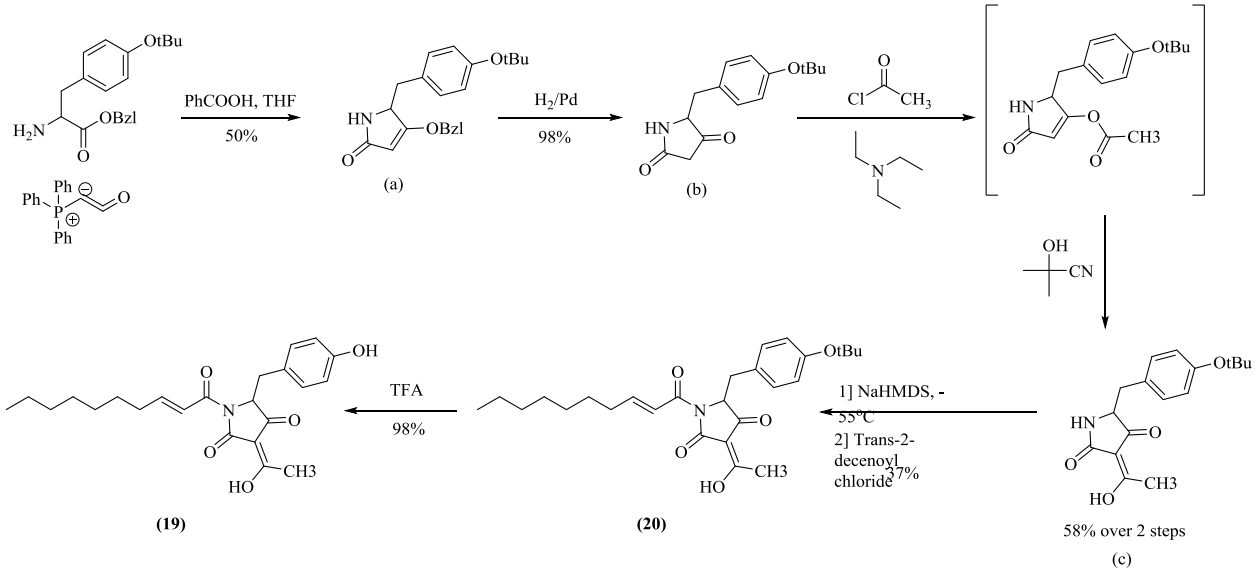
List of supplementary information

Representative synthesis procedure: Synthesis of (Z)-1-((E)-dec-2-enoyl)-5-(4-hydroxybenzyl)-3-(1-hydroxyethylidene)pyrrolidine-2,4-dione (**19**):



a] **4-(benzyloxy)-5-(4-(tert-butoxy)benzyl)-1H-pyrrol-2(5H)-one:** A mixture of benzyl 2-amino-3-(4-(tert-butoxy)phenyl)propanoate (1.428 g, 4.36 mmol), 2-(triphenylphosphoranylidene)ethenone (1.318 g, 4.36 mmol) and benzoic acid (0.107 g, 0.872 mmol) in 20ml dry THF were stirred overnight at 65°C. The solvent was evaporated and the mixture separated by column chromatography (DCM/EtOAc) to provide 770mg (50%) of product as a white solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 1.34 (s, 9H), 2.67 (dd, *J* = 13.7, 8.7 Hz, 1H), 3.17 (d, *J* = 13.7 Hz, 1H), 4.27 (d, *J* = 8.4 Hz, 1H), 4.96 (q, *J* = 11.5 Hz, 2H), 5.05 (s, 1H), 6.17 (br s, 1H), 6.92 (d, *J* = 8.4 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 2H), 7.41 (q, *J* = 7.1, 5.8 Hz, 5H). MS-ESI, m/z = 352.08 $[M+H]^{+}$

b] **5-(4-(tert-butoxy)benzyl)pyrrolidine-2,4-dione:** The 4-(benzyloxy)-5-(4-(tert-butoxy)benzyl)-1H-pyrrol-2(5H)-one (1g, 2.85 mmol) were dissolved in 30ml MeOH and 100mg (10 mol%) 10% Pd/C were added. The flask was evacuated and filled with H_2 (3x) and the

mixture stirred under H_2 (1 atm) for 2.5h. The Pd/C was filtered and rinsed with MeOH (30ml x 2). The solvent was then evaporated to provide 731mg (98%) oil which solidified on standing. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 1.24 (s, 9H), 2.46 (d, *J* = 22.1 Hz, 1H), 2.73 – 2.86 (m, 2H), 2.99 (d, *J* = 13.0 Hz, 1H), 4.15 (s, 1H), 6.84 (d, *J* = 8.1 Hz, 2H), 6.97 (d, *J* = 8.2 Hz, 2H). MS-ESI, m/z = 260.07 $[M-H]^-$

c] **(Z)-5-(4-(tert-butoxy)benzyl)-3-(1-hydroxyethylidene)pyrrolidine-2,4-dione :** Under N_2 atmosphere, to 5-(4-(tert-butoxy)benzyl)-4-hydroxy-1H-pyrrol-2(5H)-one (731 mg, 2.80 mmol) were added 15ml dry DCM followed by triethylamine (1248 µl, 8.95 mmol). The mixture was cooled in an ice bath and acetyl chloride (200 µl, 2.80 mmol) were added. UPLC analysis after 45mins indicates formation of the ester. Acetone cyanohydrin (256 µl, 2.80 mmol) was added and the mixture was stirred overnight at room temperature. The DCM was evaporated and 50ml EtOAc were added to the residue. The EtOAc layer was filtered and extracted with 5% citric acid, then brine and then dried over Na_2SO_4 and concentrated. The crude mixture was separated by reverse phase column chromatography to provide 495mg (58%) of product. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 1.36 (s, 9H), 2.49 (s, 3H), 2.63 (dd, *J* = 13.9, 10.3 Hz, 1H), 3.28 (dd, *J* = 14.1, 3.3 Hz, 1H), 4.03 (dd, *J* = 10.2, 3.4 Hz, 1H), 5.76 (br s, 1H), 6.97 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.2 Hz, 2H). MS-ESI, m/z = 302.27 $[M-H]^-$

d] **(Z)-5-(4-(tert-butoxy)benzyl)-1-((E)-dec-2-enoyl)-3-(1-hydroxyethylidene)pyrrolidine-2,4-dione (20):** To an ice cold solution of (E)-dec-2-enoic acid (427 µl, 2.308 mmol) in 4ml of dry DCM (with 2 drops of DMF) maintained under N_2 were added oxalyl chloride (208 µl, 2.423 mmol). After stirring at room temperature for 2h, the solvent was evaporated and the decenoyl chloride was dissolved in 5ml of dry THF. In another flask maintained under N_2, a solution of 3-acetyl-5-(4-(tert-butoxy)benzyl)-4-hydroxy-1H-pyrrol-2(5H)-one (350 mg, 1.154 mmol) in 15ml

of dry THF was cooled to -55°C and sodium bis(trimethylsilyl)amide (1M in THF, 508 mg, 2.77 mmol) were added. After stirring for 10mins, the decenoyl chloride in THF was added dropwise over a period of 15mins. After the addition, the temperature was allowed to rise from -55°C to -40°C over 30mins and then maintained at -40°C for another 30mins. The reaction was quenched by the addition of acetic acid (198 µl, 3.46 mmol). The solvent was evaporated and the crude mixture was separated by reverse phase chromatography to provide 196mg (37%) of product. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 0.69 (t, *J* = 6.8 Hz, 3H), 1.08 (s, 9H), 1.12 (ddd, *J* = 14.0, 7.0, 2.9 Hz, 8H), 1.32 (p, *J* = 7.3 Hz, 2H), 2.04 – 2.13 (m, 2H), 2.17 (br s, 3H), 3.03 (dd, *J* = 14.0, 2.7 Hz, 1H), 3.28 (d, *J* = 8.6 Hz, 1H), 4.57 (d, *J* = 83.7 Hz, 1H), 6.59 – 6.68 (m, 4H), 6.94 – 7.06 (m, 2H). MS-ESI, m/z = 456.59 $[M+H]^{+}$

e] **(Z)-1-((E)-dec-2-enoyl)-5-(4-hydroxybenzyl)-3-(1-hydroxyethylidene)pyrrolidine-2,4-dione (19):** A mixture of (Z)-3-acetyl-5-(4-(tert-butoxy)benzyl)-1-(dec-2-enoyl)-4-hydroxy-1H-pyrrol-2(5H)-one (100 mg, 0.219 mmol) in 4ml DCM/TFA (3:1) was stirred for 30mins. The solvent was evaporated and the crude purified by reverse phase chromatography to provide 86mg (98%) of product. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 0.90 (t, *J* = 6.6 Hz, 3H), 1.23 – 1.39 (m, 8H), 1.51 (p, *J* = 7.3 Hz, 2H), 2.30 (td, *J* = 7.3, 3.7 Hz, 2H), 2.41 (s, 3H), 3.23 (dd, *J* = 14.1, 2.7 Hz, 1H), 3.43 (dd, *J* = 14.3, 5.6 Hz, 1H), 4.78 (br s, 1H), 6.67 (d, *J* = 8.3 Hz, 2H), 6.84 (d, *J* = 8.3 Hz, 2H), 7.16 – 7.27 (m, 2H). MS-ESI, m/z = 400.29 $[M+H]^{+}$

Table S1: Activity of reutericyclin analogs against Gram-negative bacteria

Number	MIC (μg/ml) against Gram negative bacteria								
	AB	BC	EC	EC *ΔtolC*	KP	PM	PV	PA	SM
1	200	> 200	> 200	0.78	> 200	>200	>200	>200	> 200
2	>200	>200	>200	>200	>200	>200	>200	>200	>200
3	> 200	> 200	> 200	6.25	> 200	>200	>200	>200	> 200
4	> 200	> 200	> 200	1.56	> 200	>200	>200	>200	> 200
5	> 200	> 200	> 200	0.39	> 200	>200	>200	>200	> 200
6	200	> 200	> 200	100	> 200	>200	>200	>200	> 200
7	>200	> 200	>200	1.56	>200	>200	>200	>200	> 200
8	> 200	200	> 200	6.25	> 200	>200	>200	>200	200
9	50	50	25	6.25	25	200	200	25	25
10	> 200	> 200	> 200	> 200	> 200	>200	>200	>200	> 200
11	200	> 200	> 200	3.13	> 200	>200	>200	>200	200
12	>200	100	>200	25	>200	>200	>200	>200	200
13	> 200	> 200	> 200	1.56	> 200	>200	>200	>200	50
14	100	200	>200	3.13	>200	>200	>200	200	100
15	>200	>200	>200	50	>200	>200	>200	>200	>200
16	>200	>200	>200	12.5	>200	>200	>200	>200	200
17	>200	>200	>200	50	>200	>200	>200	>200	>200
18	>200	>200	>200	25	>200	>200	>200	>200	100
19	>200	>200	>200	50	>200	>200	>200	>200	100
20	>200	>200	>200	12.5	>200	>200	>200	>200	>200

Abbreviations: AB - *Acinetobacter baumannii* ATCC 19606, BC - *Burkholderia cepacia* ATCC 25416, EC - *Escherichia coli* K12, EC *ΔtolC* - *E. coli* K12 *ΔtolC*, KP - *Klebsiella pneumoniae* ATCC 33495, PM - *Proteus mirabilis* ATCC 25933, PV - *Proteus vulgaris* ATCC 33420, PA - *Pseudomonas aeruginosa* PA01, SM - *Stenotrophomonas maltophilia* ATCC 13637

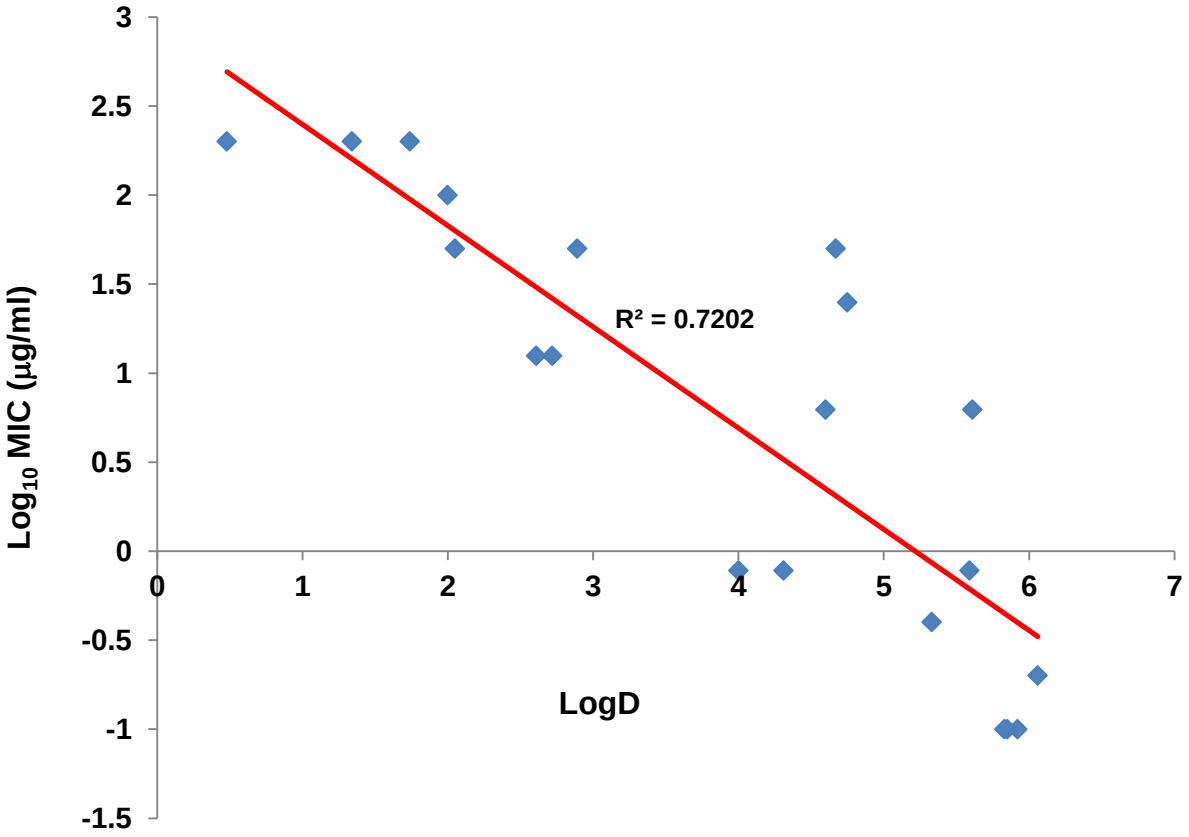


Figure S1: A plot of Log of the MICs of reutericyclins against methicillin-susceptible *S. aureus* Newman versus their lipophilicity (LogD) values shows a moderate correlation between the two parameters. For purposes of the graph, MIC values of <0.1µg/ml and >200µg/ml were considered as 0.1 and 200 respectively.

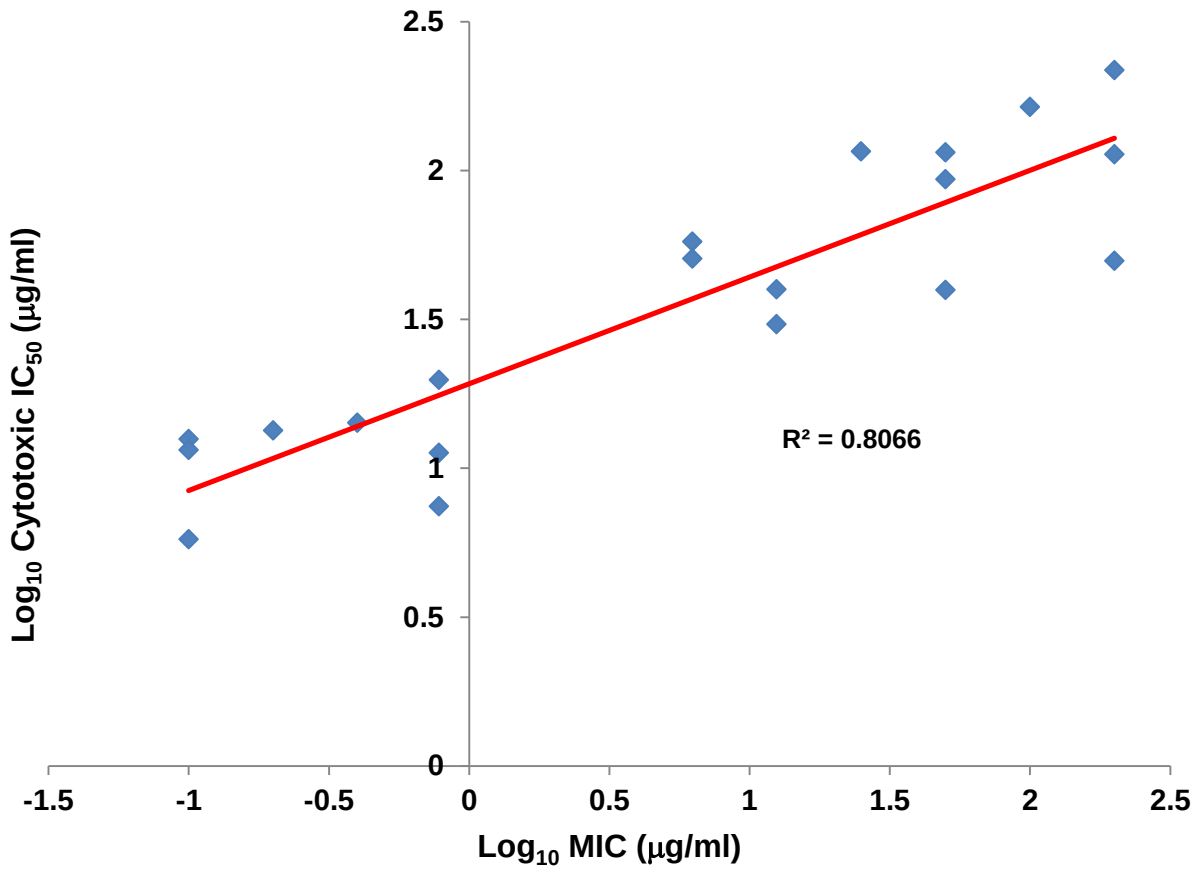


Figure S2: A plot of the Log of reutericyclins cytotoxic IC_{50} values against Vero cells versus the Log of their MICs against methicillin-susceptible *S. aureus* Newman shows a strong correlation between the two parameters. For purposes of the graph, MIC values of <0.1µg/ml and >200µg/ml were considered as 0.1 and 200 respectively.

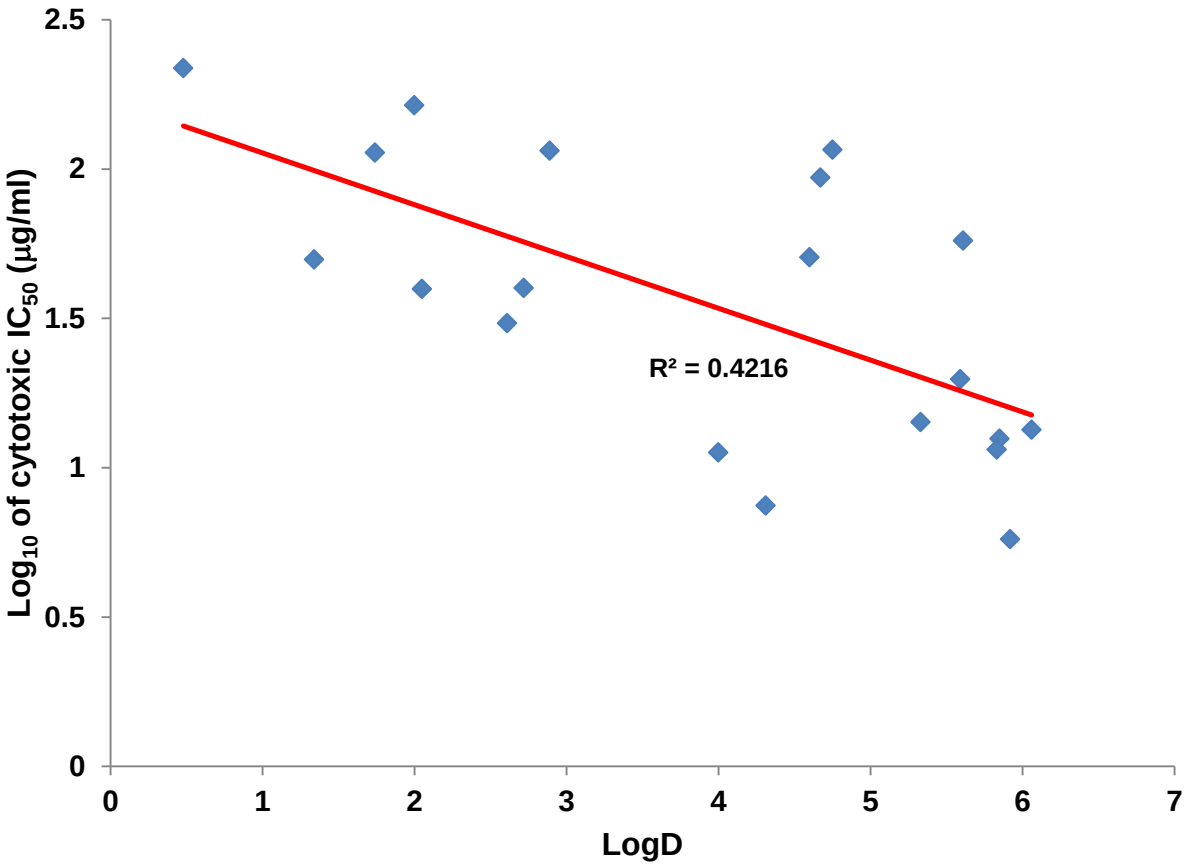


Figure S3: A plot of Log cytotoxic IC_{50} values of reutericyclins against Vero cells versus their lipophilicity (LogD) indicates a modest correlation between the two parameters.

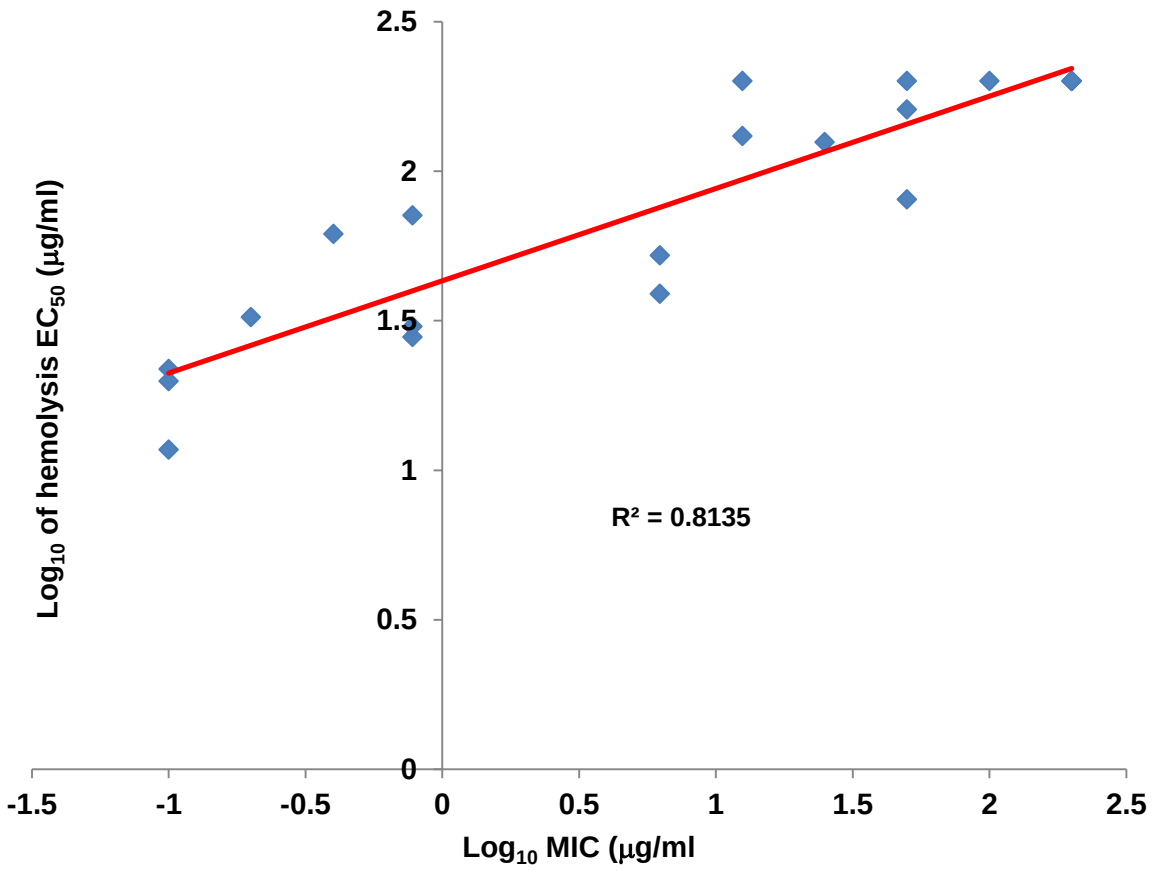


Figure S4: A plot Log of the hemolytic EC_{50} values of reutericyclins versus the Log of their MICs against methicillin-susceptible *S. aureus* Newman shows a strong correlation between the two parameters. For purposes of the graph, MIC values of <0.1µg/ml and >200µg/ml were considered as 0.1 and 200 respectively.

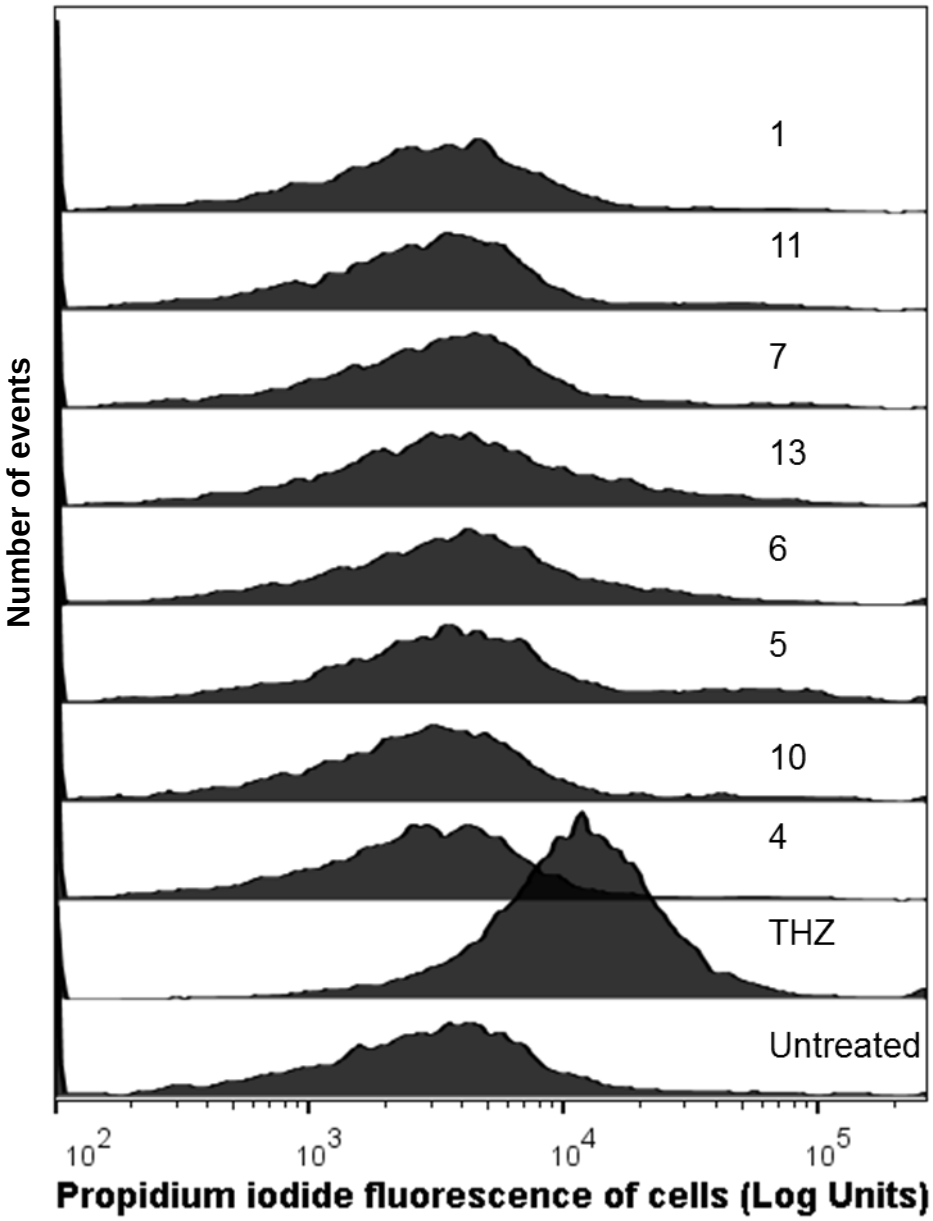


Figure S5: Effects of compounds on membrane permeability in *S. aureus* Newman as measured by changes in propidium iodide fluorescence of cells. At 10μM the reutericyclins dissipate the membrane potential without increasing membrane permeability. Thioridazine (THZ) is included as a positive control at 100μg/ml.